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Molecular detection and identification of *Rickettsia* endosymbiont in different biotypes of *Bemisia tabaci*

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INTRODUCTION

Although *Rickettsia* are the causative agents of several human and animal diseases, they are also found as endosymbionts of many invertebrates. Such symbionts maintain intimate relationships with their hosts and share common features, including intracellular localisation, maternal inheritance, and ability to influence a wide range of host traits. Others, such as *Wolbachia*, a genus related to *Rickettsia*, are known to manipulate arthropod reproduction. These abilities to manipulate the host's reproduction in their favour allow these bacteria to reach high prevalences, even fixation, within the host population, even though some of these symbionts may also harm the host, e.g., via a lessened life span [1]. Some members of *R. bellii* group are known to be vertically transmitted with induction of reproductive distortions in several insect species [2]. Conversely, host-specific pathogenic *Rickettsia* of the spotted fever and typhus groups are easily transmitted between their ticks and lice vectors to the vertebrate host with unknown effect on host vectors. This suggests evolutionary transition between different life styles, including transmission modes, beneficial or deleterious effects, manipulation of host reproduction, acquisition of new host species, evolution and emergence of vertebrate pathogenecity. To date, the way the different characteristics of *Rickettsia* have evolved remains unknown but this requires the analysis of different host-bacteria associations.

In the framework of a study on the symbiotic bacterial community associated with the major worldwide insect pest *Bemisia tabaci*, we characterised *Rickettsia* and other bacterial endosymbionts in different whitefly populations. *B. tabaci* is a species complex consisting of several genetic groups, among which are the closely related invasive B and Q biotypes. These biotypes are morphologically identical but differ in some important economic traits, such as insecticide resistance, and they are mostly recognised on mitochondrial CO1 gene sequences [3]. The Q biotype is divided into three genetic subgroups named Q1, Q2 and Q3 (or AfricaSL). All individuals harbour an obligatory symbiont and up to six different facultative bacteria, among which is a recently described *Rickettsia* spp. [4]. Here we establish the phylogenetic position of *Rickettsia* infecting *B. tabaci* and analyse correlation between bacterial distribution and variability with *B. tabaci* genetic groups to analyse the evolutionary history of the association.

METHODS

The symbiotic complex associated with the four genetic groups of *B. tabaci* was characterised using specific diagnostic PCR. RFLP-PCR and mitochondrial CO1 gene sequencing were used for host genetic group identification and phylogeny. DNA extracts were individually tested by specific primers amplifying either 16S, 23S or wsp genes of *Rickettsia*, *Cardinium*, *Hamiltonella*, *Arsenophonus*, *Fritschea* and *Wolbachia*. Precise identification of *Rickettsia* was carried out by sequencing 16S, gltA, *OmpA*, *OmpB*, *Sca1* genes.

RESULTS AND DISCUSSION

High prevalence of *Rickettsia* was detected in the B and Q2 genetic groups (90% infection in some populations) but could not be found in the Q1 and Q3 groups. Such high prevalence suggests that *Rickettsia* are able to invade a host population with different possible mechanisms that remain to be characterised in this species: (i) reproductive manipulation allowing the bacteria to spread into the host population by vertical transmission;

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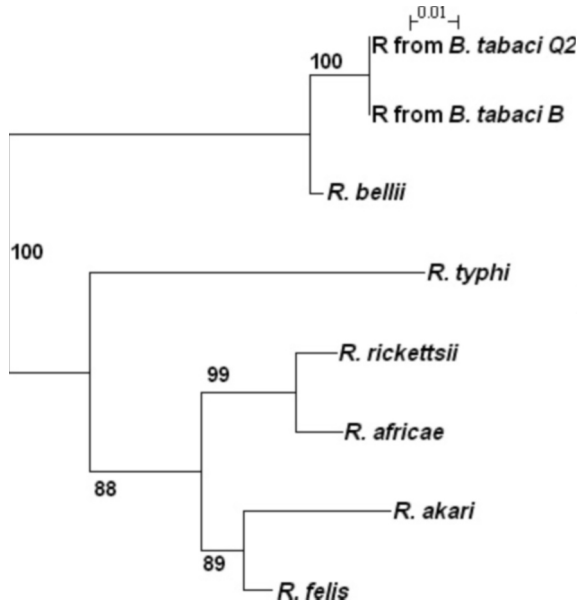

Fig. 1. Phylogenetic tree showing the relationship of *B. tabaci Rickettsia* within the genus *Rickettsia* based on concatenated *Sca1* and *gltA* genes. The tree was built by the ML method using the JTT model of protein evolution on amino acid sequences of *Sca1* and *gltA*.

(ii) horizontal transmission; (iii) hitchhiking with another spreading bacterium; or (iv) induction of a major fitness advantage. While some *Rickettsia* are known to manipulate insect reproduction by selectively killing male embryos or inducing parthenogenesis, the fact that infecting *B. tabaci* is found more often than expected together with other bacteria may hint at invasion by a hitchhiking effect. Moreover, possible horizontal transmission, as well as fitness effects of the bacterium on *B. tabaci*, are yet to be determined.

In order to identify the *Rickettsia* infecting *B. tabaci* and test whether B and Q2 genetic groups carry the same bacterium, several *Rickettsia* genes were sequenced together with the mtCO1 gene of their host. No PCR signal was detected when amplyfing *OmpA* and *OmpB* genes. The 16S rDNA places *Rickettsia* of *B. tabaci* close to *R. bellii*. This is confirmed by the *Sca1* gene (98.2% homology with *R. bellii*) and *gltA* gene (96% identity). Genetic divergence is sufficient to suggest that *B. tabaci Rickettsia*, while forming a monophyletic group with *R. belli*, probably forms a separated bacterial lineage. This is supported by 100% bootstrap value in the concatenated Sca1 and GltA gene phylogeny (Fig. 1). When comparing sequences of *Rickettsia* from B and Q2 *B. tabaci* groups, no variability was found, suggesting that the same strain of *Rickettsia* infects both biotypes. Given that B and Q2 biotypes have probably diverged over around five million years and are separated by the intermediate Q3 genetic group, which is uninfected, it seems likely that horizontal transfer of *Rickettsia* has occurred between these two groups. This hypothesis is supported by the fact that B and Q2 are found in the same geographic region in the Mediterranean Middle-East and *Rickettsia* distribution in the whole body of insects probably favours such transfers [5]. An alternative hypothesis could be that the presence of *Rickettsia* is an ancestral state that has been lost in Q1 and Q3 groups.

Genbank accession numbers for the *gltA* and *Sca1* sequences obtained in this study are EU760764, EU760765, EU760766 and EU760767.

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